



**UNIVERSITI PUTRA MALAYSIA**

**EXPERIMENTAL TRIAL ON EFFICACY OF CLOXACILLIN AS DRY  
COW THERAPY AND 0.5% IODINE AS PREMILKING AND  
POSTMILKING TEAT DIPPING TO REDUCE BOVINE MASTITIS**

**RABINDRA THAKUR**

**FPV 2004 3**

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**By**

**RABINDRA THAKUR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Veterinary Science**

**March 2004**



## **DEDICATION**

**This Thesis is dedicated to my**

- late grand father Sri Ram Swarup Thakur, late grand mother, Ram Shakhi,
- father Sri Bibhishan Thakur, mother Ram Snehi
- wife, Veena and children Piyush, Pallavi , Paritosh, Shreya and Utkarsh,
- father-in-law, Prof. Arun Kumar Arun, mother- in -law, Ram Rati,
- brother, Dharendra, sister, Rekha, sister in law Mamta and brother in law Sri Arun Kumar Sinha and Sri Santosh Kumar

**for their full support and encouragement**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Veterinary Science

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**March 2004**

**Chairman: Siti Zubaidah Ramanoon**

**Faculty: Veterinary Medicine**

The efficacy of dry cow therapy, pre and postmilking teat dipping for bovine mastitis was studied. A total of 54 Holstein-Friesian late pregnant dairy cows on one farm in their third to tenth lactation were selected. Animals were randomly allocated to treatment and untreated control groups. At drying off, treatment group received 500mg cloxacillin as dry cow therapy (DCT), intramammarily. Iodine 0.5% as teat dip was applied at pre and postmilking. The DCT cured infected quarter, significantly ( $P < 0.05$ ) for Any organisms (Gram-negative and Gram-positive) and *Staphylococcus aureus*. Prevention of mastitis using DCT was not significant ( $P > 0.05$ ).

Self cure rates at cow level was 16%-50% and at quarter level was 16%-65% for Any organism, *S. aureus*, coagulase-negative *staphylococcus* and Other organism (Other than *S. aureus* and CNS), respectively. New infection rates at cow level were 0%-75% and at quarter level was 18%-32%. Teat dipping did not significantly prevent new cases of mastitis ( $P > 0.05$ ). The California mastitis test (CMT) positive was

significantly reduced ( $P<0.05$ ) in treated quarters. The mean colony forming unit (cfu)  $\log_{10}$  was  $2.88 \pm 0.32$  (mean  $\pm$  standard error of the mean) in first month after calving, significantly lower ( $P<0.05$ ) in comparison to dry off and control group ( $5.76 \pm 0.24$  and  $5.63 \pm 0.37$ ), respectively. The percent efficacy of the DCT was 48.8%. In conclusion, DCT significantly cured infected quarters and teat dipping did not prevent new cases of mastitis.

Keywords: Cow, mastitis, dry cow therapy, teat dip.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

**EKSPERIMEN PERCUBAAN UNTUK MENGUJI KEMUJARABAN  
CLOXACILLIN SEBAGAI TERAPI PENGERINGAN LEMBU DAN  
PENGUNAAN 0.5% IODIN SEBAGAI CELUPAN PUTING SUSU  
SEBELUM DAN SELEPAS PERAHAN SUSU UNTUK MENGURANGKAN  
MASTITIS LEMBU**

**Oleh**

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Satu kajian ke atas mastitis lembu telah dijalankan untuk menguji kemujaraban terapi pengeringan lembu dan celupan puting susu sebelum dan selepas pemerahan susu. Sebanyak 54 ekor lembu tenusu Holstein–Friesian, bunting berat, dalam laktasi ketiga hingga sepuluh, telah dipilih daripada sebuah ladang. Lembu tenusu dibahagikan secara rawak kepada kumpulan rawatan dan kawalan. Pada masa pengeringan, kumpulan rawatan diberi cloxacillin 500mg secara intramamari. Puting susu dicelup dengan 0.5% Iodin sebelum dan selepas pemerahan susu. Terapi pengeringan lembu terbukti dapat menyembuhkan jangkitan sukuan ambing susu untuk kategori sebarang organisma (Gram-negatif dan Gram-positif) dan *Staphylococcus aureus*. Pencegahan mastitis dengan menggunakan terapi pengeringan lembu didapati tidak bekesan ( $P>0.05$ ).

Kadar penyembuhan sendiri dalam lembu ialah 16%-50% dan sukuan ialah 16%-65% bagi sebarang organisma, *S. aureus*, koagulasi-negatif *staphylococcus* dan Organisma lain. Kadar jangkitan baru dalam lembu ialah 0%-75% dan dalam sukuan ialah 18%-32%. Celupan puting susu tidak dapat mencegah jangkitan baru mastitis dengan berkesan ( $P > 0.05$ ). Ujian California mastitis positif didapati berkurangan dengan berkesan ( $P < 0.05$ ) dalam sukuan yang dirawat. Purata unit pembentukan koloni (cfu)  $\log_{10}$  ialah  $2.88 \pm 0.32$  (purata  $\pm$  sisihan piawai purata) dalam bulan pertama selepas beranak adalah masing –masing kurang ( $P < 0.05$ ) berbanding semasa tempoh kering dan kumpulan kawalan ( $5.76 \pm 0.24$  dan  $5.63 \pm 0.37$ ). Peratus kemujaraban bagi terapi pengeringan lembu adalah 48.8%. Kesimpulannya, terapi pengeringan lembu adalah berkesan menyembuhkan jangkitan sukuan ambing susu dan celupan puting susu tidak dapat mencegah jangkitan baru mastitis.

Kata kunci: Lembu, mastitis, terapi pengeringan lembu, celupan puting susu.

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I certify that an Examination Committee met on 16<sup>th</sup> March 2004 to conduct the final examination of Rabindra Thakur on his Master of Veterinary Science thesis entitled “Experimental trial on efficacy of cloxacillin as dry cow therapy and 0.5% iodine as premilking and postmilking teat dipping to reduce bovine mastitis” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

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## LIST OF ABBREVIATIONS

ADH	Arginine Di-Hydrolase
Any organism	All Gram-negative and Gram-positive organism
CFU	Colony forming unit
$\chi^2$	Chi-square test
CMT	California mastitis test
CIR	Cow infection rate
CNS	Coagulase-negative <i>staphylococcus</i>
DCT	Dry cow therapy
ELISA	Enzyme linked immunosorbent assay
Epi Info	Epidemiology Information
FOECC	Fluoro-opto-electric-cell-counting
FET	Fisher exact test
IMI	Intramammary infection
IPT	Indicator paper test
MDG	Methyl- alpha- D- Glucopyranoside
NAGase	N- acetyl- $\beta$ -D-glucosaminadase
NaOH	Sodium hydroxide
NMC	National Mastitis Council
Other organism	Organism other than <i>S. aureus</i> and CNS
QIR	Quarter infection rate
SA	<i>Staphylococcus aureus</i>
SCC	Somatic cell count
SCM	Subclinical Mastitis
SE	Standard error
SPSS	Statistical package for social services
URE	Urease
VP	Voges-proskauer
WMT	Wisconsin mastitis test

## CHAPTER 1

### INTRODUCTION

Bovine mastitis is generally characterized by inflammation of the parenchyma of the mammary gland in the presence of significantly increased leukocyte content in milk (Radostits *et al.*, 2000). Causes of mastitis may be by physical, chemical, managemental, mycotic and primarily bacteriological and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, pus, the presence of clots, abnormal secretion in the gland and presence of a large numbers of leukocytes. There is swelling, heat, pain, fever, marked depression, rapid weak pulse, sunken eyes, weakness and complete anorexia (Radostits *et al.*, 2000).

Many infective agents cause intramammary infections (IMI). The common microorganisms are *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli* (<http://www.nmconline.org>). Risk factors for mastitis are infected quarters, teat injury, efficiency of milking personnel, milking speed and hygiene in the milk parlor. Susceptibility of the cow to mastitis was found to be related to stage of lactation, early (50 days) post calving (Sargeant *et al.*, 1998), age of cow, lactation number (more than four lactations) (Emanuelson and Funke, 1991), level of inherited resistance, teat shape, anatomy of teat canal, teat lesions (especially the orifice), distance from ground to teat, immunological factors including leukocyte and status of each mammary gland including prior infection (Radostits *et al.*, 2000).

The risk of IMI is increased with high milking rate and large teat canal diameter. It is also associated with increased somatic cell count (SCC) (Slettbakk *et al.*, 1995), lack of vitamin E, A and selenium (Jukola *et al.*, 1996; Weiss *et al.*, 1997).

The economic losses from mastitis occur in the form of reduced milk production and quality, shortened reproductive life, premature culling of the affected animals, and veterinary costs for treatment. It has been estimated that mastitis results in severe economic losses of around US\$182 per cow annually. The total annual losses due to mastitis in U.S.A. are about US\$2 billion (Miller *et al.*, 1993). A single infected gland results in a loss of 770 kg of milk per cow (McDonald, 1979).

The widespread use of mastitis control measures such as: (1) milking machine maintenance, (2) teat dipping, (3) early treatment of clinical cases, (4) dry cow therapy, (5) culling of cows with chronic mastitis (<http://www.nmconline.org>) has led to considerable progress in controlling mastitis caused by contagious pathogens such as *S. agalactiae* and *S. aureus*. Nevertheless, environmental mastitis has become a major problem in many well-managed dairy farms (Oliver and Mitchell, 1984; Smith *et al.*, 1985a; Oliver, 1988; Booth, 1988; Todhunter *et al.*, 1995; Milne *et al.*, 2002).

Milking machine has direct contact with teat and it transports milk from teat to processing plant. The milking machine can influence new IMI from one animal to the other. It is crucial to understand the basic components, functions, and operation of the milking equipment, maintenance and importance of good milking technique (Spencer, 1989).

Premilking udder preparation and teat sanitation is necessary to reduce the microbial population and minimize new IMI. It may be performed by washing and drying with a single service paper towel. Predipping and udder preparation have a significant effect on milk bacterial counts, and on the incidence of mastitis (Ruegg and Dohoo, 1997). Predipping controls the growth of environmental mastitis. Postmilking teat dipping immediately after every milking with a germicidal solution reduce (contagious pathogen) the incidence of new udder infections by 50-90% (Boddie and Nickerson, 1997).

Early treatment of the clinical cases alleviates clinical signs, achieves a bacteriological cure and restores the cow's production. In addition, it also limits the spread of infection, eradicate a specific pathogen, and increases herd production (Radostits *et al.*, 2000).

Dry cow therapy (DCT) eliminates the existing infections by 70-98%. Cure rate varies according to organism for coagulase-negative *staphylococcus* (CNS) over 95% (Davidson *et al.*, 1994) and *S. aureus* 85.2% (Pankey *et al.*, 1982a). Prolong use of DCT eliminates *S. agalactiae* by 90-100%. Dry cow therapy reduces the incidence of new IMI by 50-75% (Williamson *et al.*, 1995). The cure rate of *S. aureus* IMI varies from 40 to 83% (Rindsig *et al.*, 1978; Ziv *et al.*, 1981).

Culling of chronically infected cow can help to eliminate the existing IMIs. Animals which are infected with *S. aureus* may have fibrosis in the mammary epithelium and the infection persists for long time. These animals are poorly responding to antimicrobial therapy. Sometimes 4-5 cases of clinical mastitis occur in current

lactation. Therefore, chronically infected animals should be culled (Stott and Kennedy, 1993).

Conventional method for bacteriological isolation and identification is used worldwide. California mastitis test is an indirect test which is a rapid and practical test to determine approximate somatic cell concentration in milk (Miller and Kearns, 1967). It is also in use since a long time for mastitis diagnosis. It has been considered as a reference method in mastitis diagnosis.

Among the environmental pathogens, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Enterobacteriae* spp. are the most prevalent, infecting mammary glands as favourable conditions arise (Smith *et al.*, 1985b; Oliver, 1988; Todhunter *et al.*, 1995; Milne *et al.*, 2002).

The prevalence of IMI from pathogens is about 50% of cows and quarter infection rate of about 25% (Fox *et al.*, 1995). The prevalence of infection in dairy heifers of breeding age and in pregnant dairy heifers varies widely from 30-50% (Fox *et al.*, 1995) and 18% of quarters (Pankey *et al.*, 1991) to as high as 97% of heifers and 75% of quarters (Nickerson *et al.*, 1995). The average annual incidence of mammary infection of clinical quarter cases per 100 cows at risk per year including the dry period in individual herds ranges from 10-12% (Miltenburg *et al.*, 1986) but higher values, ranging from 16-65% in some herds (Firat, 1993; Bartlette *et al.*, 1992b).

Studies on the effect of premilking, postmilking teat dipping and DCT have not been reported in Malaysia. Evaluation of DCT, pre and postmilking teat dipping by

comparing colony forming unit in treatment and control group has also not been performed. Therefore, this study evaluates the efficacy of DCT, pre and postmilking teat dipping in bovine mastitis.

This study aims to determine the efficacy of Noroclox ® (Cloxacillin 500mg, Norbrook Laboratories Limited U.K.) as a DCT in eliminating existing IMI and preventing new IMI and to determine the efficacy of 0.5% iodine as a pre and postmilking teat dip in bovine mastitis.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

Mastitis is the most common disease affecting dairy cows worldwide. Fifty percent of the cows may be infected in one or more quarters (Ziv, 1992). It affects milk production, loss of glandular secretory tissue, systemic illness, and cause death. Mastitis has many effects on raw milk components, milk product quality and yield. There is a decrease in the production of lactose, fat, nonfat milk solids, and casein. Whey proteins, sodium, chloride, pH, and free fatty acids are increased. Total protein may remain stable as increases in albumin and immunoglobulins offset the decrease in casein. Inflammatory cells (somatic cells) are increased.

Mastitis has 38% morbidity (Sischo, 1990). On an annual basis, three of every 10 dairy cows have clinical inflammation of the mammary gland. Seven percent are culled and 1% dies as a consequence of the disease.

Mastitis may result from the introduction of microorganisms through the teat sphincter. The clinical course of the disease varies with the ability of bacteria to colonize and thrive in the mammary gland secretions, their inherent virulence, and the type of magnitude, and duration of the host response to the bacterial invasion. The inflammation of the mammary gland is indicated by clinical signs (Radostits *et al.*, 2000).